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A pre-clinical evaluation of silver, iodine and Manuka honey based dressings in a model of traumatic extremity wounds contaminated with *Staphylococcus aureus*

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ABSTRACT

Prevention of extremity war wound infection remains a clinical challenge. *Staphylococcus aureus* is the most common pathogen in delayed infection. We hypothesised that choice of wound dressings may affect bacterial burden over 7 days reflecting the current practice of delayed primary closure of wounds within this timeframe.

A randomised controlled trial of 3 commercially available dressings (Inadine® (Johnson & Johnson, NJ, USA), Acticoat® (Smith & Nephew, Hull, UK), Activon Tulle (Advancis Medical, Nottingham, UK)) was conducted in a rabbit model of contaminated forelimb muscle injury. A positive control group treated with antibiotics was included. Groups were compared to a saline soaked gauze control. The primary outcome was a statistically significant reduction ($p < 0.05$) in tissue *S. aureus* at 7 days post-injury. Secondary outcome measurements included bacteraemias, observational data, whole blood determination, ELISA for plasma biomarkers, PCR array analysis of wound healing gene expression and muscle/lymph node histopathology.

Antibiotic, Inadine and Acticoat groups had statistically significant lower bacterial counts (mean 7.13 [95% CI 0.00–96.31] $\times 10^2$; 1.66 [0.94–2.58] $\times 10^5$; 8.86 [0.00–53.35] $\times 10^4$ cfu/g, respectively) and Activon Tulle group had significantly higher counts (2.82 [0.98–5.61] $\times 10^6$ cfu/g) than saline soaked gauze control (7.58 [1.65–17.83] $\times 10^5$ cfu/g). There were no bacteraemias or significant differences in observational data or whole blood determination. There were no significant differences in muscle/loss or pathology and lymph node cross-sectional area or morphology. There were some significant differences between treatment groups in the plasma cytokines IL-4, TNFα and MCP-1 in comparison to the control. PCR array data demonstrated more general changes in gene expression in the muscle tissue from the Activon Tulle group than the Inadine or Acticoat dressings with a limited number of genes showing significantly altered expression compared to control.

This study has demonstrated that both Acticoat® and Inadine® dressings can reduce the bacteria burden in a heavily contaminated soft tissue wound and so they may offer utility in the clinical setting particularly where surgical treatment is delayed.

Introduction

Over 400 UK military and civilian personnel have been killed as a result of hostile action in Afghanistan since 2001 [1] and many more have been injured. The majority of these casualties are the result of the improvised explosive devices used ubiquitously by insurgents in recent conflicts [2,3]. The extremities are the most commonly injured anatomical regions in war accounting for between half [2] and two thirds of all wounds [3]. Most of these

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extremity wounds are penetrating soft tissue wounds but around a quarter involve a fracture; of which the vast majority are open fractures [2]. Studies of high-energy, complex and heavily contaminated wounds have identified high rates of infectious complications [4,5] and eventual osteomyelitis [6] which may be a result of the initial contamination or subsequent contamination during staged evacuation and treatment at medical facilities of increasing sophistication.

The microflora of war wounds evolves over time [7] and historical studies have demonstrated both seasonal [8] and geographic [9–11] variation. During the recent conflicts in Iraq and Afghanistan it is become clear that Gram-positive organisms dominate the initial contamination [12] and largely reflect bacterial carriage in the population at risk [13], Gram-negative organisms are then more prevalent in early wound colonization [14,15] or primary infection [16,17] and finally *Staphylococcus aureus* is identified in the majority of delayed infectious complications [15] which result in further surgery, delayed rehabilitation and worse functional outcome.

Frequently war wounds are anatomically complex and seen in physiologically vulnerable servicemen whose evacuation to the next level of care (where further surgical debridement can be carried out) cannot be guaranteed due to ongoing hostilities. For these reasons military surgeons cannot rely on a single surgical debridement, however expertly performed, to eradicate all initial bacterial contamination and of course prevent subsequent contamination. Therefore the evidence based use of adjuncts to surgery, such as wound dressings, is an important area of research and practice. Given the heterogenous nature of war wounds and the presence of multiple confounding variables such as poly-microbial contamination, multiple injury, haemorrhagic shock, massive blood transfusion and the effect of blast injury we believe that a clinical randomized controlled trial of war wound dressings would be extremely challenging to perform and may not answer the research question.

In order to investigate the effect of wound dressings on this most clinically significant microorganism the Defence Science and Technology Laboratory first developed an animal model of a *S. aureus* contaminated extremity war wound [18] and then conducted a 48 h randomized controlled trial of antimicrobial wound dressings [19]. This trial reproduced the common scenario where contamination persists despite current management strategies and assessed whether the 'residual' bacterial load could be reduced by dressings alone and whether there was any injurious effect associated with their use.

The aim of the current trial was to assess the antimicrobial efficacy of commercially available wound dressings which have all been used or are being considered for use by the UK military by extending the time frame to reflect the current clinical practice of delayed primary closure of clean viable war wounds at around 5–7 days after injury [20–22]. We tested the hypothesis that wounds treated with gauze dressings impregnated with iodine (Inadine®), silver (Acticoat®) or Manuka honey (Activon Tulle) will have significantly lower *S. aureus* counts after 7 days than a control group treated with saline soaked gauze dressings. In addition, we evaluated the potential harmful effects of the dressings via assessment of plasma biomarkers and tissue pathology and molecular biology.

Materials and methods

Study design and ethics

This trial was conducted under a license issued by the UK Home Office under the authority of the Animals (Scientific Procedures) Act 1986. A local ethical review board approved the work and 3R's principals were adhered to. A power calculation based on the earlier trial suggested that 6 animals in each group would be sufficient to detect a statistically significant difference in bacterial count, which was the primary outcome in this study. Secondary outcome measurements included bacteraemia, animal behaviour, weight change, temperature, whole blood determination, plasma and tissue markers of inflammation and repair and muscle and lymph node histopathology.

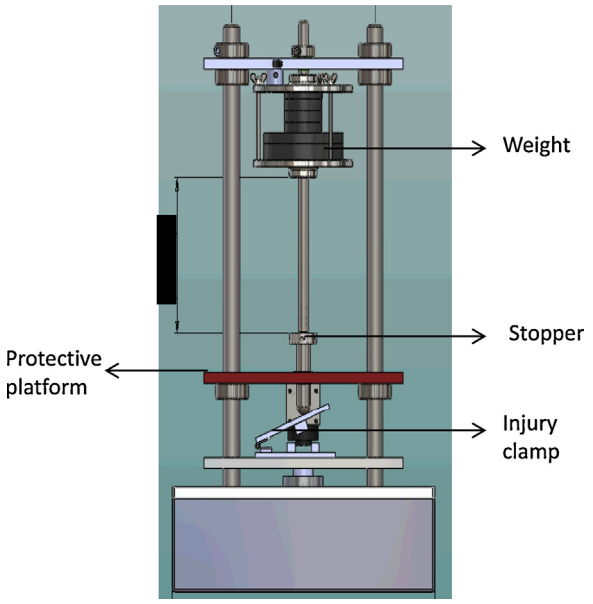


Fig. 1. Muscle injury jig. The isolated muscle is held between the jaws of the injury clamp. The weight held in place by an electronically controlled magnet on release the weight falls to the level of the stopper which pushed down on the clamp. The protection plate and design of the injury clamp ensure only the isolated muscle is injured.

Surgical, injury and microbiological technique

A detailed description of the surgical and microbiological methodology has been reported previously by this group [18].

Briefly, groups of skeletally mature female New Zealand White (NZW) rabbits (3.87 kg, 4.73–3.12 kg) were randomized to each of 5 test groups.

Under general anaesthesia the flexor carpi ulnaris (FCU) muscle belly was surgically isolated and injured using the bespoke jig (Fig. 1).

Following injury the FCU muscle belly was directly inoculated with a challenge dose of 10^6 colony forming units (CFU) of *S. aureus* (National Collection of Type Culture 4163) using a pipette.

A subcutaneous injectable non-steroidal anti-inflammatory was administered (4 mg/kg Carprofen – Rimadyl, Pfizer, Sandwich, UK) and repeat doses were administered daily for the remainder of the study. (This drug was given to reduce the post-injury pain associated with traumatic injury. Reducing the incidence of lameness with the use of an analgesic reduced the overall severity of the procedure as well as ensuring that adverse effects such as infection, if present, was more easily determined.)

A subcutaneous Implantable Programmable Temperature Transponder (IPTT, Biomedic Data Systems Inc., Delaware, USA) was inserted between the scapulae.

The rabbits remained anaesthetized and monitored for 3 h prior to application of randomized test dressing (described below) and a support bandage was applied prior to recovery from anaesthesia.

Twice daily temperature and behaviour scores for each animal were recorded.

In contrast to the previous study at 2 days post injury the animals underwent a dressing change under anaesthesia.

As previously described at the end of the experiment (7 days post injury in this instance) the animals were sacrificed by overdose of intravenous Phenobarbital sodium (Dolethan 200 mg/ml, Vetoquinol, Buckingham, UK) and post-mortem blood and tissue samples were retrieved. Tissue samples included the FCU muscle belly, skin and axillary lymph nodes from both the injured and contralateral limbs to facilitate comparative analysis. The FCU muscle belly was divided longitudinally and sent for histopathological and quantitative microbiological assessments (bacterial counts).

Wound swabs were taken immediately post-injury, 3 h post inoculation, at the 2 day dressing change and on completion of the trial. These swabs were processed using a plate-spread technique. Additionally post mortem blood samples were incubated in nutrient broth (Oxoid, Cambridge, UK) and sub-cultured.

Homogenized centrifuged muscle samples were analyzed using a modified serial dilution and drop plate technique described by Herigstad [23]. This modification of the serial dilution and drop plate technique was also used to verify the challenge dose. All samples, wound swabs, homogenized muscle and blood were incubated on no-selective blood agar and selective chromogenic agar plates (*S. aureus* ID (SAID) bioMerieux UK Ltd., Basingstoke, UK).

Dressings

Inadine® (Johnson & Johnson, NJ, USA), Acticoat® (Smith & Nephew, Hull, UK), Activon Tulle (Advancis Medical, Nottingham, UK), sterile saline (Sodium Chloride 0.9% (w/v) Aquapharm No1, Animalcare Ltd., York, UK) soaked gauze. A further sterile saline soaked gauze dressing group was additionally provided daily subcutaneous Enrofloxacin 10 mg/kg (Baytril, Bayer Corporation, Newbury, UK) to act as a positive control group.

Histopathology

Tissue samples were fixed in 10% neutral buffered formalin, dehydrated through graded alcohols, cleared in chloroform and xylene and impregnated with paraffin wax. Specimens stained with haematoxylin and eosin were examined using light microscopy (AxioScope, Carl Zeiss, Welwyn Garden City, UK) and images captured with a digital camera (AxioCam, Carl Zeiss, Welwyn Garden City, UK).

The severity of muscle necrosis/loss was subjectively assessed by comparing the stained sections from the injured limb with the corresponding sections from the uninjured limb with score of 0 to 4 recorded (0 = not present, 1 = <25%, 2 = 25–50%, 3 = 50–75%, 4 = >75%). A subjective semi-quantitative scoring criterion was developed to grade pathology in muscle. The incidence of haemorrhage, inflammatory cell infiltration and muscle tissue fibrosis was recorded (0 = not present, 1 = moderate or 2 = severe).

The 'Magic Wand' tool of photoshop (Adobe, San Jose, USA) was used to determine the size of lymph nodes from digital images of 600 dpi resolution acquired from stained lymph node sections using a flatbed scanner (Hewlett Packard, Palo Alto, USA). Lymph node morphology was scored using a subjective semi-quantitative criteria based on degree of lymphocyte proliferation through the regions of the lymph nodes (0 = normal, 1 = lymphocyte proliferation within well defined primary nodules, 2 = proliferation beyond primary follicles into cortex, 3 = proliferation throughout LN (primary follicles indistinct from cortex, but medullary regions still well defined), 4 = extensive lymphocyte proliferation throughout cortex and medullary regions – regions indistinct).

Haematology

Whole blood was collected into Ethylenediaminetetra-acetic acid (EDTA) coated tubes for whole blood determination (Advia 120 with multi-species function, Siemens Healthcare, Frimley, UK) prior to surgery, 3 h post-injury, at the 2 day dressing change and on completion of the study at 7 days.

Molecular biology

At 7 days post-injury whole blood was collected into Lithium Heparin tubes for assessment of biomarkers in plasma by ELISA. After mixing the blood was centrifuged and the supernatant (plasma) removed and stored at −80 °C until needed. ELISAs were carried out (duplicate wells per sample) as per the manufacturer's instructions (Cusabio Ltd., Hubei Province, China). Plates were read at 450 nm with a reference reading taken at 540 nm.

For PCR array analysis of wound healing gene expression post-mortem muscle samples (approx. 0.5 cm^3) were placed into 1 ml RNAlater™ (Ambion Inc.) and stored as per the manufacturer's instructions. RNA was extracted using a hybrid Trizol®/Qiagen RNeasy® protocol. Total RNA was quantified using a Nanodrop 1000 and treated with turbo DNase (Ambion Inc.) as per the manufacturer's instructions whereby its quantity and quality was again checked using NanoDrop 1000 and Bioanalyser (Bio-rad). All RIN numbers were >8. The target A260/280 ratio was >1.8 and <2.1 and the target A260/230 ratio was >1.7. Samples outside of this range were cleaned using ethanol precipitation. Subsequently, 2 μg RNA was shipped to SABiosciences™ (Hilden, Germany) for reverse transcription using the RT^2 First Strand Kit (SABiosciences™) followed by the samples being run on the rabbit wound healing RT^2 Profiler™ PCR array (PANZ-121, SABiosciences™). Data analysis was conducted using the RT^2 Profiler™ PCR array Excel data analysis tool (SABiosciences™).

Statistical analysis

All samples and data were analyzed by personnel blinded to the dressings used in each group. Statistical analysis was performed using Minitab 16 (Mintab Inc., USA). Microbiology data were normalized using a recognized transformation process and the mean difference between challenge dose and retrieved bacterial counts for each group compared to the control using 2-way ANOVA to allow for the observed effect of time as well as the test dressing. Plasma cytokines levels at 7 days were compared to the control using 1-way ANOVA with Dunnett's post-test. Non-parametric histology data were compared to the control using a Kruskal–Wallis test. A p value of <0.05 was considered significant for all statistical tests. For the PCR array, data analysis was carried out using the RT^2 Profiler™ PCR array Excel data analysis tool (SABiosciences™). All five reference genes on the plate were used for normalization. The injured muscle gene expression data for all the dressings were compared to the levels in the control dressing (saline) and the results were displayed on a volcano plot with a 2-fold change in gene expression and $p < 0.05$ being considered significant.

Results

Microbiology

The primary outcome of this study was muscle tissue quantitative microbiology at 7 days post-injury. The initial power

Table 1
The number of positive *S. aureus* cultures at each stage of qualitative microbiology – figures in parentheses show how many of the positive results were noted to be present in low numbers.

	2 day swab	7 day swab
Control (saline soaked gauze)	5	5 (1)
Positive control (antibiotics and saline soaked gauze)	3 (2)	1 (1)
Inadine (iodine)	5	4
Activon Tulle (Manuka honey)	5	5
Acticoat (silver)	5	2

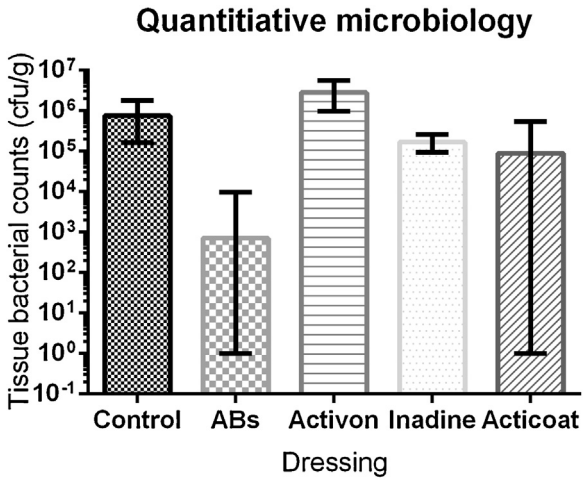


Fig. 2. Forelimb muscle tissue bacterial counts at 7 days post-injury. Mean with 95% confidence interval.

calculation predicted a sample size of 6 animals per group however an interim analysis determined that $n = 5$ was sufficient to establish statistical significance for each group.

One animal in the control group cultured a contaminant at 2 days, this animal was excluded from the study and repeated. Therefore, results from 25 of the 26 animals are included with $n = 5$ in each group.

The positive control, Inadine and Acticoat groups all had statistically significant lower bacterial counts (mean 7.13 [95% CI 0.00–96.31] $\times 10^2$; 1.66 [0.94–2.58] $\times 10^5$; 8.86 [0.00–53.35] $\times 10^4$ cfu/g, respectively) and the Activon Tulle group had significantly higher bacterial counts (2.82 [0.98–5.61] $\times 10^6$ cfu/g) than the saline soaked gauze control (7.58 [1.65–17.83] $\times 10^5$ cfu/g).

There were no bacteria identified on any of the immediate post-injury wound swabs confirming the cleanliness of the equipment. All wound swabs taken at 3 h post-inoculation grew *S. aureus*. Results for wound swabs at 2 and 7 days are shown below (Table 1).

There were no bacteraemias identified on any of the blood cultures taken at 7 days.

Quantitative microbiology results are shown below (Fig. 2).

Histopathology

There was extensive muscle loss by comparison to the uninjured contralateral FCU muscle in all treatment groups but no significant difference in the extent of this between groups. There was no significant difference between groups in the muscle pathology observed with all samples showing evidence of haemorrhage, inflammation and/or necrosis. All samples of injured muscle tissue displayed collagen deposition and fibrosis/granulation tissue formation at the periphery of the site of the initial injury. All groups had higher cross sectional areas of ipsilateral axillary lymph nodes by comparison to nodes taken from the contralateral side. However, there were no significant differences in cross sectional area of lymph nodes between groups. Nor was there a significant difference in lymph node morphology between groups.

Observational

There were no differences in animal behaviour between groups. No animals appeared to be in significant distress throughout the study.

Haematology

Neutrophil counts and platelets were elevated above the upper limit of the normal range in all groups (data not shown).

Molecular biology

There were no appreciable levels of TGFβ, C-reactive protein (CRP), procalcitonin (PCT), or IL-1 in the plasma at 7 day post-injury. Appreciable levels of IL-4, IL-6, MCP-1 and TNFα could be measured at the end of the study. There were no significant differences in IL-6 plasma level between the groups (data not shown). In contrast, animals in the Activon Tulle group had significantly higher levels of plasma IL-4 and TNFα compared to the saline control ($p < 0.05$, Fig. 3a and b). Animals treated with

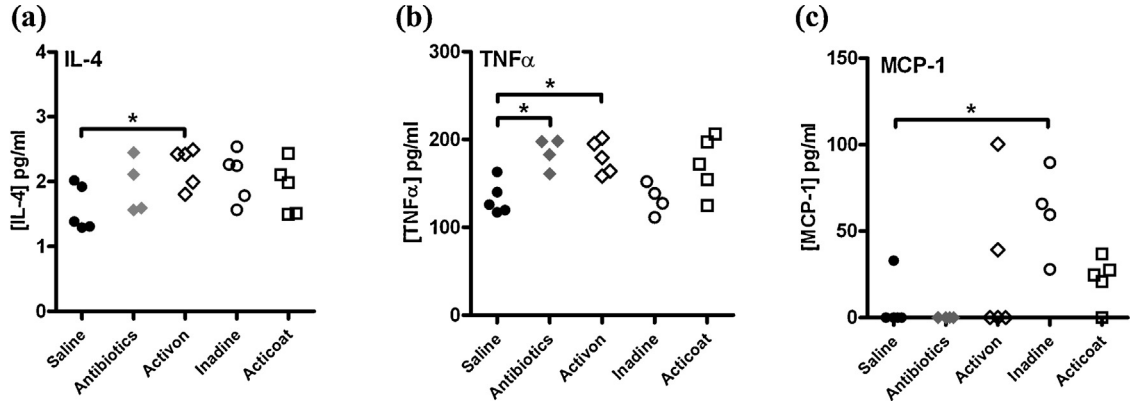
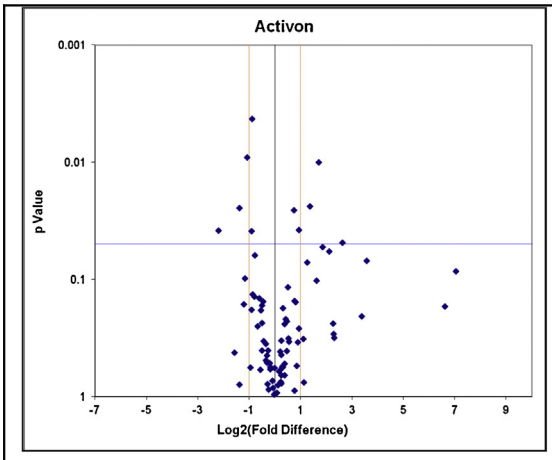
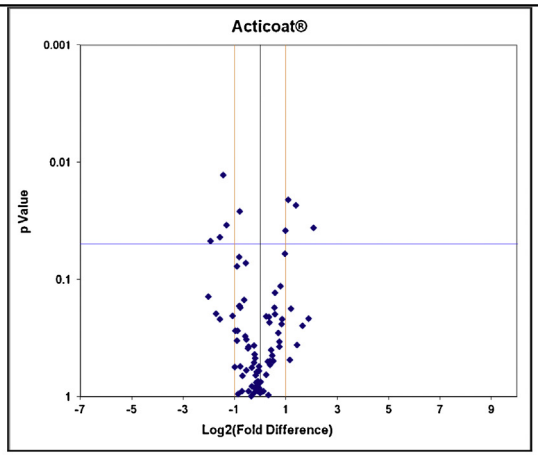


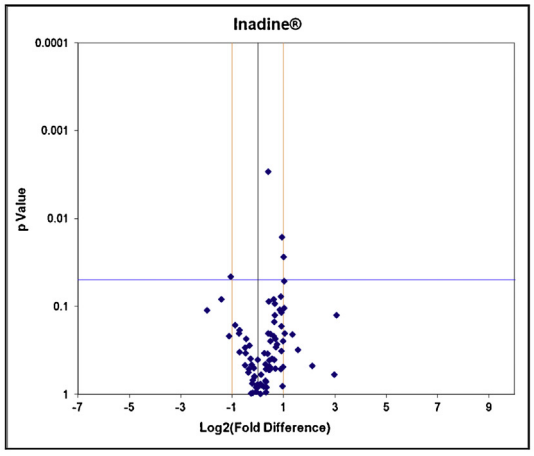
Fig. 3. IL-4 (a), TNFα (b) and MCP-1 (c) concentrations in plasma at 7 days post-infection as measured by ELISA. * denotes $p < 0.05$ one-way ANOVA with Dunnett's post-test compared to saline dressing.





Gene	Fold change	p value
CD40L	6.19	0.049
Tissue Factor	3.27	0.010
COX-2	2.57	0.024
PLAU	-2.13	0.009
IGF1	-2.60	0.025
WNT2	-4.59	0.038

Gene	Fold change	p value
IL-2	4.20	0.036
β-catenin	2.60	0.023
WNT1 inducible signaling pathway protein 3	2.14	0.021
Collagen, type I, α2	-2.50	0.034
Tenascin C	-2.73	0.013
MCP-1	-3.00	0.044
WNT2	-3.81	0.047



Gene	Fold change	p value
Integrin α6	2.00	0.027
Collagen, type I, α2	-2.08	0.046

Fig. 4. Gene expression levels of 81 genes in muscle tissue from the injured/infected limb (right) in the Activon, Acticoat® and Inadine® compared to saline control. Genes demonstrating significantly different expression to the saline control group are shown in.

antibiotics also had significantly higher levels of plasma TNFα than the saline control ($p < 0.05$, Fig. 3b). MCP-1 was undetectable in the plasma of animals treated with antibiotics and all bar one of the animals in the saline control group. Levels of plasma MCP-1 were consistently higher in animals treated with the iodine-soaked dressing ($p < 0.05$, Fig. 3c).

The RT^2 Profiler™ rabbit wound healing PCR array (SABiosciences™) was used to investigate changes in the expression of genes associated with stages in the wound healing pathway. The results were displayed in a volcano plot which maps out all 81 genes on the array and their log2 fold difference (x-axis) from control. This plot also encompasses the statistical significance of the results by plotting p value (y-axis). Gene expression levels were measured in muscle tissue from the injured/infected limb (right) (Fig. 4) and the uninjured limb (left) (data not shown). As expected, levels of all the markers were significantly lower in the uninjured leg muscle compared to the injured muscle. The genes achieving statistical significance for each dressing are detailed in a table below each graph.

The array data demonstrates that the Activon dressing has caused more general changes in gene expression than the Inadine or Acticoat dressings.

Discussion

This study used an animal model of a *S. aureus* contaminated extremity muscle injury to investigate the anti-microbial efficacy of commercially available wound dressings. The primary outcome

was quantitative microbiology of the muscle at 7 days post-injury. The study demonstrated that iodine (Inadine®) and silver (Acticoat®) dressings applied for 7 post-injury (with a dressing change at 48 h post-injury) resulted in a significant reduction in the number of bacteria recovered at 7 days compared to saline soaked gauze control. However, the magnitude of the effect of the dressings was not as great as the administration of systemic antibiotics where the bacterial counts were zero in 4 of the 5 animals. The study also demonstrated that a Manuka honey dressing (Activon Tulle) had minimal, if any, anti-bacterial activity with significantly greater bacteria present at 7 days compared to saline controls.

The longer duration of this current study compared to the previous study by this group (7 versus 2 days) allowed evaluation of the clinical significance of the difference in microbiological load. This evaluation was undertaken using histopathological analysis of muscle tissue and molecular biological analysis of blood and muscle tissue however no clear differences were seen between groups.

The design of the study reflects the clinical scenario. The extremities are the most common anatomical regions wounded in war and *S. aureus* is the most clinically relevant micro-organism encountered at the time of initial contamination and later infection. The high-energy crush injury and inoculation with 10^6 cfu *S. aureus* models the moment of contaminated battlefield injury. The 3-h delay to application of a dressing duplicates the clinical timelines to evacuation and first surgical treatment currently being achieved in Afghanistan. The dressing change at 2 days represents the clinical scenario whereby seriously wounded servicemen are evacuated back to the UK within 48 h and their wounds are urgently reassessed in an operating theatre. The end point of the study after 7 day reflects current UK military practice of delayed primary closure of war wounds at 5–7 days after injury.

The dressings selected for inclusion in this trial have all been used or are being considered for use by the UK military. They have demonstrated antibacterial activity in other studies but there is a paucity of literature regarding their use in acute wounds and the existing literature reports variable efficacy [24–29] and/or toxicity [30–35]. The clinical use of povidine iodine is still contentious and a recent WHO report on hand hygiene has indicated contaminated iodine antiseptic preparations have resulted in outbreaks of pseudomonas infection [36]. A RCT reported that healing with Inadine was no worse than either Aquacel or a simple non-adherent dressing in diabetic patients with venous leg ulcers [37]. Additionally, Vermeulen et al. [38] have reviewed RCTs that have reported iodine use and whilst most of the RCTs are old they concluded that there was no evidence to support that iodine dressings reduced wound healing. However there are several reports from *in vitro* and animal studies demonstrated toxicity or slowed healing [39–42].

It has been proposed that Manuka honey dressings are effective due to the high osmolarity created by its sugar content, the hydrogen peroxide it releases as the honey comes into contact with wound exudates and the effect of poorly understood phytochemicals [24]. Compounds and dressings containing free iodine are effective against bacteria through a combination of intra-cellular effects on protein, nucleotide and free fatty acid structure and function. In an oxygen charged aqueous media bulk silver acts as a catalyst for destructive oxygenation of microorganisms [43] and a study investigating the mechanism of action of silver ions revealed intracellular deposition of silver containing electron-dense granules associated with loss of DNA replication and detachment of the cytoplastic membrane from the cell wall [44]. Acticoat contains nanocrystalline silver, this formulation of silver is reported to increase surface area to volume ratios and result in sustained release of silver cations with low tissue concentrations of silver.

As a model of extremity war wounds this study does have some limitations. In order to achieve reproducibility and ethical acceptability it was not possible to use high-energy penetrating trauma as a mode of injury, the injury was simulated using the bespoke jig. The study was powered to detect statistically significant differences in bacterial counts at 7 days and therefore it is likely that the group size is one of the limiting factors for the interpretation of the histopathology and molecular biology. The other limiting factor in this regard was the relatively short duration of the study and a study of wound healing may require a study length of at least 14 days. Another limitation to the study and interpretation of results is the lack of a non-injured control group and an injured but non-contaminated control group. Inclusion of these additional control groups would have been interesting and may have added to the interpretation of the histopathology and molecular biology. These additional groups were not necessary for interpretation of the primary outcome and so were not included to ensure adherence to the principal of the 3R's, using the minimum number of animals to achieve the objective of the study. A recent study has highlighted the difficulties of performing a randomized controlled trial (RCT) in the most relevant population [45]. Although the authors should be commended for undertaking the study it demonstrates the need for pre-clinical models to evaluate potential therapeutic strategies for extremity trauma as patient recruitment was one of the limiting factors in the study.

In the Acticoat group the bacterial counts ranged from 6.4×10^2 cfu/g to 7.4×10^5 cfu/g without any clear outliers. The small group size does not permit definitive analysis of data within each group but if the bactericidal effect of silver nanocrystals relies on fairly intimate contact with the dressing (as this dressing is intended to produce low tissue concentrations of free silver cations which are readily taken up by chloride ions in wound exudates) then one might reasonably expect this dressing to be less effective where bacteria are deep within more complex war wounds. It has been demonstrated that in burn wounds treated with silver sulfadiazine that most of the silver is associated with the superficial eschar and very little is absorbed into deeper layers [46,47]. It may be useful to study the silver concentration in different layers of a traumatic wound dressed with Acticoat in a similar way.

Tissue healing and repair following injury is important to maximise functional recovery post-injury. Wound healing may be complicated if there is bacterial contamination and so in this study evaluation of blood and muscle tissue was undertaken to try and elucidate whether healing was improved in those animals with reduced bacterial counts. There were some statistically significant results between groups but the results did not demonstrate that any dressing was superior to another in terms of promoting an appropriate inflammatory and repair response.

The molecular biology results from the Activon group demonstrate greater inflammation [48,49] and reduced regeneration [50–53] compared to the control group. This may be due to increased bacterial burden in this group. Although the higher plasma IL-4 in the Activon Tulle group compared to control is contradictory [54].

The gene analysis from the Acticoat® group indicated a switch from inflammation (downregulation of MCP-1) to wound healing [55–57]. It is not possible to determine whether these effects are directly mediated by the silver ions within the dressing, or are due to the reduced bacterial burden, it is perhaps a combination of the two.

The Inadine® group showed very few significant gene expression changes and thus interpretation of results is difficult. The

chemokine MCP-1 was significantly raised in the plasma compared with saline. Raised systemic levels of MCP-1 have also been shown to be associated with the development of heterotropic ossification following penetrating trauma [58]. It was not possible to determine the significance of the changes in plasma cytokines and tissue gene expression between the groups. The primary outcome of the study was quantitative microbiology at 7 days post-injury and so it was not possible to follow the animals to full healing. The authors acknowledge that such a study would be necessary to evaluate whether the changes indicating poor healing versus repair resulting in actual differences in time to healing and this should be a consideration for future studies.

Further work in this area could assess the effect of different contaminating bacteria in isolation and/or in combination as war wounds are often contaminated with and colonized by several species of bacteria. Future studies could develop the wound model further either (1) to extend the study timeline to facilitate the evaluation of dressings and/or other therapies on wound healing or (2) to include a fracture and consider the effect of different modes of fracture stabilization on outcome. These follow-on studies are currently an aspiration. Other institutions such as United States Army Institute of Surgical Research have been active in this field and have evaluated other wound therapies in different pre-clinical models [59–64] and results from these studies must be evaluated prior to continuation and development of the current rabbit model.

Conclusion

This study in a small animal model of *S. aureus* contaminated muscle injury demonstrated that both Inadine® and Acticoat® significantly reduced the tissue bacterial load compared to a saline soaked gauze dressing. The use of these dressings could be considered as an adjunct to surgical debridement and systemic antibiotics for the treatment of contaminated injuries both on and off the battlefield. The use of such anti-microbial dressings may be particularly useful when delays in surgery are anticipated such as extended evacuation times. In addition, the study has shown that in this model Activon Tulle has minimal anti-bacterial activity and so its use in a heavily contaminated wound is not recommended.

Conflicts of interest statement

None of the authors have any financial or personal relationships with other people or organisations that could inappropriately influence (bias) their work.

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References

[1] Defence Mo. British Fatalities Operations in Afghanistan. https://www.gov.uk/government/fields-of-operation/afghanistan2013.
[2] Owens BD, Kragh Jr JF, Macaitis J, Svoboda SJ, Wenke JC. Characterization of extremity wounds in Operation Iraqi Freedom and Operation Enduring Freedom. J Orthop Trauma 2007;21(4):254–7.
[3] Ramasamy A, Harrisson S, Lasrado I, Stewart MP. A review of casualties during the Iraqi insurgency 2006 – a British field hospital experience. Injury 2009;40(5):493–7.
[4] Murray CK, Wilkins K, Molter NC, Yun HC, Dubick MA, Spott MA, et al. Infections in combat casualties during Operations Iraqi and Enduring Freedom. J Trauma 2009;66:S138–44.
[5] Petersen K, Riddle MS, Danko JR, Blazes DL, Hayden R, Tasker SA, et al. Trauma-related infections in battlefield casualties from Iraq. Ann Surg 2007;245(5):803–11.
[6] Brown KV, Murray CK, Clasper JC. Infectious complications of combat-related mangled extremity injuries in the British military. J Trauma 2010;69(1):S109–15.
[7] Fleming A. On the bacteriology of septic wounds. Lancet 1915;638–43.
[8] Lindberg R, Wetzler T, Marshall J, Newton A, Strawitz J, Howard J. The bacterial flora of battle wounds at the time of primary debridement: a study of the Korean battle casualty. Ann Surg 1955;141:369–74.
[9] Altemeier W. Bacteriology of traumatic wounds. J Am Med Assoc 1944;127(7):413–7.
[10] Klein RS, Berger SA, Yekutiel P. Wound infection during the Yom Kippur war: observations concerning antibiotic prophylaxis and therapy. Ann Surg 1975;182(1):15–21.
[11] Kovaric J, Matsumoto T, Dobek A, Hamit H. Bacterial flora of one hundred and twelve combat wounds. Mil Med 1968;622–4.
[12] Murray CK, Roop SA, Hospenthal DR, Dooley DP, Wenner K, Hammock J, et al. Bacteriology of war wounds at the time of injury. Mil Med 2006;171(9):826–9.
[13] Yun HC, Murray CK, Roop SA, Hospenthal DR, Gourdine E, Dooley DP. Bacteria recovered from patients admitted to a deployed U.S. military hospital in Baghdad, Iraq. Mil Med 2006;171(9):821–5.
[14] Murray CK. Epidemiology of infections associated with combat-related injuries in Iraq and Afghanistan. J Trauma 2008;64(3 Suppl.):S232–8.
[15] Yun HC, Branstetter JG, Murray CK. Osteomyelitis in military personnel wounded in Iraq and Afghanistan. J Trauma 2008;64(2 Suppl.):S163–8.
[16] Johnson EN, Burns TC, Hayda RA, Hospenthal DR, Murray CK. Infectious complications of open type III tibial fractures among combat casualties. Clin Infect Dis 2007;45(4):409–15.
[17] Simchen E, Sack T. Infection in war wounds: experience during the 1973 October war in Israel. Ann Surg 1975;182(6):754–61.
[18] Eardley WG, Martin KR, Taylor C, Kirkman E, Clasper JC, Watts SA. The development of an experimental model of contaminated muscle injury in rabbits. Int J Low Extrem Wounds 2012;11(4):254–63.
[19] Eardley WG, Martin KR, Clasper JC, Watts SA. The efficacy of antimicrobial wound dressings used in the management of military complex extremity injury: a pre-clinical randomised controlled trial. Br J Surg 2011;98:S2.
[20] Brown KV, Guthrie HC, Ramasamy A, Kendrew JM, Clasper J. Modern military surgery: lessons from Iraq and Afghanistan. J Bone Joint Surg Br 2012;94(4):536–43.
[21] Giannou C, Baldan M. Delayed primary closure and skin grafting. In: Giannou C, Baldan M, editors. War surgery. Working with limited resources in armed conflict and other situations of violence, vol. 1. International Committee of the Red Cross; 2010.
[22] Guthrie HC, Clasper JC, Kay AR, Parker PJ. Initial extremity war wound debridement: a multidisciplinary consensus. J R Army Med Corps 2011;157(2):170–5.
[23] Herigstad B, Hamilton M, Heersink J. How to optimize the drop plate method for enumerating bacteria. J Microbiol Methods 2001;44(2):121–9.
[24] Drosou A, Falabella A, Kirsner RS. Antiseptics on wounds: an area of controversy. Wounds 2013;15(5):149–66.
[25] Gethin G, Cowman S. Manuka honey vs. hydrogel – a prospective, open label, multicentre, randomised controlled trial to compare desloughing efficacy and healing outcomes in venous ulcers. J Clin Nurs 2009;18(3):466–74.
[26] Jull A, Walker N, Parag V, Molan P, Rodgers A. Randomized clinical trial of honey-impregnated dressings for venous leg ulcers. Br J Surg 2008;95(2):175–82.
[27] Molan PC, Betts JA. Clinical usage of honey as a wound dressing: an update. J Wound Care 2004;13(9):353–6.
[28] Visavadia BG, Honeysett J, Danford MH. Manuka honey dressing: an effective treatment for chronic wound infections. Br J Oral Maxillofac Surg 2008;46(1):55–6.
[29] Willix DJ, Molan PC, Harfoot CG. A comparison of the sensitivity of wound-infecting species of bacteria to the antibacterial activity of Manuka honey and other honey. J Appl Bacteriol 1992;73(5):388–94.
[30] Atiyeh BS, Costagliola M, Hayek SN, Dibo SA. Effect of silver on burn wound infection control and healing: review of the literature. Burns 2007;33(2):139–48.
[31] Cooper ML, Laxer JA, Hansbrough JF. The cytotoxic effects of commonly used topical antimicrobial agents on human fibroblasts and keratinocytes. J Trauma 1991;31(6):775–82.
[32] Fong J, Wood F. Nanocrystalline silver dressings in wound management: a review. Int J Nanomed 2006;1(4):441–9.
[33] Hill KE, Malic S, McKee R, Rennison T, Harding KG, Williams DW, et al. An in vitro model of chronic wound biofilms to test wound dressings and assess antimicrobial susceptibilities. J Antimicrob Chemother 2010;65(6):1195–206.
[34] Smoot III EC, Kucan JO, Roth A, Mody N, Debs N. In vitro toxicity testing for antibacterials against human keratinocytes. Plast Reconstr Surg 1991;87(5):917–24.
[35] Trop M, Novak M, Rodl S, Hellbom B, Kroell W, Goessler W. Silver-coated dressing acticoat caused raised liver enzymes and argyria-like symptoms in burn patient. J Trauma 2006;60(3):648–52.
[36] World Health Organisation. WHO Guidelines on hand hygiene in health care; 2009.
[37] Jeffcoate WJ, Price PE, Phillips CJ, Game FL, Mudge E, Davies S, et al. Randomised controlled trial of the use of three dressing preparations in the management of chronic ulceration of the foot in diabetes. Health Technol Assess 2009;13(54):1–86. iii–iv.

[38] Vermeulen H, Westerbos SJ, Ubbink DT. Benefit and harm of iodine in wound care: a systematic review. J Hosp Infect 2010;76(3):191–9.
[39] Arai K, Yamazaki M, Maeda T, Okura T, Tsuboi R. Influence of various treatments including povidone-iodine and healing stimulatory reagents in a rabbit ear wound model. Int Wound J 2013;10(5):542–8.
[40] Thomas GW, Rael LT, Bar-Or R, Shimonkevitz R, Mains CW, Slone DS, et al. Mechanisms of delayed wound healing by commonly used antiseptics. J Trauma 2009;66(1):82–90. discussion 90–1.
[41] van Meurs SJ, Gawlitta D, Heemstra KA, Poolman RW, Vogely HC, Kruyt MC. Selection of an optimal antiseptic solution for intraoperative irrigation: an in vitro study. J Bone Joint Surg Am 2014;96(4):285–91.
[42] Wilson JR, Mills JG, Prather ID, Dimitrijevich SD. A toxicity index of skin and wound cleansers used on in vitro fibroblasts and keratinocytes. Adv Skin Wound Care 2005;18(7):373–8.
[43] Davies RJ, Etris SF. Development and functions of silver in water-purification and disease control. Catal Today 1997;36:107–14.
[44] Feng QL, Wu J, Chen GQ, Cui FZ, Kim TN, Kim JO. A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*. J Biomed Mater Res 2000;52(4):662–8.
[45] Fries CA, Ayalew Y, Penn-Barwell JG, Porter K, Jeffrey SLA, Midwinter MJ. Prospective randomised controlled trial of nanocrystalline silver dressing versus plain gauze as initial post-debridement management of military wounds on wound microbiology and healing. Injury 2014;45(7):1111–6.
[46] Harrison HN. Pharmacology of sulfadiazine silver. Its attachment to burned human and rat skin and studies of gastrointestinal absorption and extension. Arch Surg 1979;114(3):281–5.
[47] Tsipouras N, Rix CJ, Brady PH. Passage of silver ions through membrane-mimetic materials, and its relevance to treatment of burn wounds with silver sulfadiazine cream. Clin Chem 1997;43(2):290–301.
[48] Ashcroft GS, Jeong MJ, Ashworth JJ, Hardman M, Jin W, Moutsopoulos N, et al. Tumor necrosis factor-alpha (TNF-alpha) is a therapeutic target for impaired cutaneous wound healing. Wound Repair Regen 2012;20(1):38–49.
[49] Gulotta LV, Kovacevic D, Cordasco F, Rodeo SA. Evaluation of tumor necrosis factor alpha blockade on early tendon-to-bone healing in a rat rotator cuff repair model. Arthroscopy 2011;27(10):1351–7.
[50] Adams PD. Healing and hurting: molecular mechanisms, functions, and pathologies of cellular senescence. Mol Cell 2009;36(1):2–14.
[51] Clemmons DR. Role of IGF-1 in skeletal muscle mass maintenance. Trends Endocrinol Metab 2009;20(7):349–56.
[52] Lluis F, Roma J, Suelves M, Parra M, Aniorte G, Gallardo E, et al. Urokinase-dependent plasminogen activation is required for efficient skeletal muscle regeneration in vivo. Blood 2001;97(6):1703–11.
[53] Pelosi L, Giacinti C, Nardis C, Borsellion G, Rizzuto E, Nicoletti C, et al. Local expression of IGF-1 accelerates muscle regeneration by rapidly modulating inflammatory cytokines and chemokines. FASEB J 2007;21(7):1393–402.
[54] Varin A, Gordon S. Alternative activation of macrophages: immune function and cellular biology. Immunobiology 2009;214(7):630–41.
[55] Hurvitz JR, Suwairi WM, Van HW, El-Shanti H, Superti-Furga A, Roudier J, et al. Mutations in the CCN gene family member WISP3 cause progressive pseudorheumatoid dysplasia. Nat Genet 1999;23(1):94–8.
[56] Schutze N, Schenk R, Fiedler J, Mattes T, Jakob F, Brenner RE. CYR61/CCN1 and WISP3/CCN6 are chemoattractive ligands for human multipotent mesenchymal stroma cells. BMC Cell Biol 2007;8:45.
[57] Yeger H, Perbal B. The CCN family of genes: a perspective on CCN biology and therapeutic potential. J Cell Commun Signal 2007;1(3–4):159–64.
[58] Evans KN, Forsberg JA, Potter BK, Hawksworth JS, Brown TS, Anderson R, et al. Inflammatory cytokine and chemokine expression is associated with heterotropic ossification in high-energy penetrating trauma. J Orthop Trauma 2012;26(11):204–13.
[59] Penn-Barwell JG, Murray CK, Wenke JC. Comparison of the antimicrobial effect of chlorhexidine and saline for irrigating a contaminated open fracture model. J Orthop Trauma 2012;26(12):728–32.
[60] Penn-Barwell JG, Murray CK, Wenke JC. Local antibiotic delivery by a bioabsorbable gel is superior to PMMA Bead Depot at Reducing Infection in an open fracture model. J Orthop Trauma 2013. August 14 [Epub ahead of print].
[61] Stinner DJ, Hsu JR, Wenke JC. Negative pressure wound therapy reduces the effectiveness of traditional local antibiotic depot in a large complex musculoskeletal wound animal model. J Orthop Trauma 2012;26(9):512–8.
[62] Stinner DJ, Noel SP, Haggard WO, Watson JT, Wenke JC. Local antibiotic delivery using tailorable chitosan sponges: the future of infection control? J Orthop Trauma 2010;24(9):592–7.
[63] Stinner DJ, Waterman SM, Masini BD, Wenke JC. Silver dressings augment the ability of negative pressure wound therapy to reduce bacteria in a contaminated open fracture model. J Trauma 2011;71(1 Suppl.):S147–50.
[64] Wenke JC, Guelcher SA. Dual delivery of an antibiotic and a growth factor addresses both the microbiological and biological challenges of contaminated bone fractures. Expert Opin Drug Deliv 2011;8(12):1555–69.